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Ortho-Aminoacetophenone, A Non-Lethal Repellent: The Effect of Volatile Cues *vs.* Direct Contact on Avoidance Behavior by Rodents and Birds

Shirley A. Wager-Pagé* & J. Russell Mason

US Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center, c/o Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104 USA

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Abstract: Taxonomic differences in responsiveness to chemosensory irritants are prevalent among avian and mammalian species and represent a major obstacle to the development of general vertebrate repellents. We evaluated the effect of *ortho*-aminoacetophenone (OAP), a potent avian repellent, on ingestive behavior of two rodent species, Prairie vole (*Microtus ochrogaster*), Deer Mouse (*Peromyscus maniculatus* Wagn.), and an avian species, European starling (*Sturnus vulgarus* L.) utilizing similar experimental conditions to facilitate inter-species comparisons. All three species avoided OAP-treated food. Apple consumption by voles was decreased from a baseline of theoretical zero% by OAP (0·01–10·0 ml liter^{-1}), $P < 0{\cdot}00001$, while mice avoided all but the lowest concentration of OAP (0·01–10 ml liter^{-1}), $P < 0{\cdot}00001$. A repellent should elicit avoidance behavior prior to the animal having physical contact with the commodity, ideally producing aversion *via* volatile cues rather than through direct contact. Therefore, we utilized two delivery methods for presentation of the test solutions to evaluate the repellency of OAP in the presence and absence of direct contact. Apple consumption by birds following exposure to OAP by either direct contact or volatile cues differed from a baseline of theoretical zero% consumption, $P < 0{\cdot}001$. When birds had access to OAP through both direct and volatile exposures, reduction in apple consumption by European starlings was greater than observed following contact with the compounds volatile cues alone, $P < 0{\cdot}03$. These findings argue against a major role for olfaction or naso-trigeminal chemoreception in avoidance of OAP-treated food. Instead, taste or oral trigeminal chemoreception appear to mediate responding.

Key words: chemosensory, irritant, repellent, taxonomic differences, avian, rodent.

1 INTRODUCTION

Birds and mammals often differ in their responsiveness to chemosensory irritants.[1] Birds rarely avoid chemosensory irritants that mammals typically find aversive although they are able to detect them.[2] Rock dove (*Columba livia* Gmelin), red-winged blackbird (*Agelaius phoeniceus* L.), European starling (*Sturnus vulgaris* L.) and gray partridge (*Perdix perdix* L.) are indifferent to ammonia.[3,4] Parrots (*Amazona* spp.), rock doves,[5] red-winged blackbirds,[6] European starlings,[7] house finches (*Cadopacus mexicanus*),[8] and cedar waxwings (*Bombycilla cedrorum* Vieill)[8] are indifferent to high (1000 ppm) concentrations of capsaicin, the active ingredient in capsicum peppers. Mammals typically avoid much lower concentrations of these substances: 100 ppm capsaicin is a strong irritant to rodents.[9] European starlings and red-winged blackbirds also are insensitive to gingerol and piperine,[3] the active ingre-

* To whom correspondence should be addressed.

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dients in ginger and black pepper, respectively. This taxonomic difference in responsiveness is of practical significance because of growing interest in non-lethal repellent chemicals that can be used for bird and mammal control.

Ortho-aminoacetophenone (OAP) may represent a useful bird and mammal repellent.[7,10–12] Aminoacetophenones are effective avian repellents in both feeding[7] and drinking[10] contexts. Although some evidence suggests that aminoacetophenones also repel rodents,[11,12] other studies indicate that rodents are relatively insensitive to these substances.[13] Differences in experimental design make direct comparisons between taxa difficult. The present studies were designed to evaluate the effect of varied concentrations of OAP on ingestive behavior of prairie voles, deer mice and European starlings in similar feeding contexts.

2 EXPERIMENTAL

2.1 Experiment 1

2.1.1 Subjects

Adult male prairie voles (*Microtus ochrogaster;* 37–47 g) and male and female deer mice, (*Peromyscus maniculatus* Wagn.; 18–28 g), (n = 10/group) were randomly selected from the Monell colonies and individually caged in wire-topped plastic boxes (17·8 × 29·2 × 12·7 cm). All animals were given aspen shavings as bedding and held under a 12:12 h light:dark cycle in rooms at 23°C. Purina rabbit lab chow #5321 (voles) and rodent diet #8604 (mice) and tapwater were available *ad libitum*.

2.1.2 Test materials

Ortho-aminoacetophenone (CAS No. 551-93-9; Sigma Chemical Company, St. Louis, MO) was dissolved in 100% ethanol to produce a stock solution which was mixed with vegetable oil to produce the following concentrations: 0·001, 0·1, 1·0, and 10·0 ml liter^{-1}. The solution for control coatings consisted of ethanol diluted in vegetable oil in the same proportions utilized in preparation of the OAP solutions, hereafter referred to as oil. Preweighed apple pieces were coated with the OAP and oil solutions to produce test foods.

2.1.3 Procedure

Animals were given two weeks to adapt to experimental conditions before the studies began. To determine baseline consumption, each animal was presented with two apple pieces coated with oil solution on each of two pre-treatment days. The apple pieces were placed in opposite corners at the front of each cage. Following a two-hour test period, the remainder of each piece of apple was weighed to estimate consumption. Two-hour consumption of apple pieces coated with oil plus ethanol was compared to data obtained from a pilot study in which voles were presented with oil-coated apple pieces (no ethanol). It was determined that the small quantities of ethanol present in the oil vehicle did not alter two-hour apple intake compared to oil only (data not shown).

The pre-treatment period was followed by a treatment period during which one piece of apple was coated with an OAP solution and the other with oil. Each experiment was performed in duplicate and presentation positions of the OAP- and oil-coated apples were counterbalanced to compensate for potential side preferences. In these studies each animal was presented with only one of the four OAP concentrations to avoid carryover or learning effects. Owing to the small number of deer mice available, one group was tested with two concentrations of OAP (0·1, 1·0 ml liter^{-1}) separated by a three-week rest period.

2.1.4 Analysis

Difference scores were calculated by subtracting consumption of OAP-coated apple pieces from consumption of oil-coated apple pieces and expressing as percentages of control intake. These data were then tested to determine whether the intake of OAP-coated apple differed from a theoretical baseline value of zero% ingestion in a two-way analysis of variance (ANOVA). A minor modification in the calculation of the treatment sums of squares was made by utilization of the theoretical zero% as the baseline, and the degrees of freedom reflect the additional level in concentrations considered in the analysis. This modification does not change the overall analysis, since zero% of baseline intake has been substituted for control apple consumption which was incorporated into the calculation of the difference scores. Dunnett's tests[14] were used to isolate significant differences between baseline and treatment means. $P < 0.05$ was considered statistically significant.

2.2 Experiment 2

Experiment 1 showed that OAP was repellent to mice and voles. The second experiment was designed to explore the importance of volatile cues in the observed avoidance response.

2.2.1 Subjects

Nine experimentally naive prairie voles were selected from the Monell colony and individually caged and maintained as described in Section 2.1.1. Test materials were prepared as described in Section 2.1.2.

2.2.2 Procedures

Following a two-week adaptation period, all animals were exposed to untreated apple pieces in the presence of the odor of OAP (10 ml liter^{-1}) or oil in no-choice tests. For odor exposure, Whatman #3 filter paper discs (2·3 cm diam) (Fisher Scientific Corp., NJ) were

saturated with OAP (10 ml liter^{-1}) or oil solutions. For control trials, the filter paper was saturated with OAP or oil solution and encased in plastic mesh histoprep cassettes, (Fisher Scientific Corp., NJ).[15] The cassettes were attached to the middle of the lower half of the front wall of each cage with pre-weighed oil-coated apple pieces placed directly in front of them. After two hours had elapsed, the remaining apple was weighed to determine consumption. Each vole was presented with OAP and control odors twice in four separate no-choice tests. Test sessions were conducted twice a week with at least one day elapsing between test periods.

For comparative purposes, voles were also presented with oil- and 10 ml liter^{-1} OAP-coated apple slices in no-choice tests to evaluate the relative effects of volatile *vs.* direct contact + volatiles on avoidance behavior. On test days, treatment or control conditions, apple slices were placed at the center front of each cage and consumption was measured after two hours.

2.2.3 *Analysis*

To compare avoidance of OAP's volatile cues when presented in histoprep cassettes with the effects of direct contact with OAP coating on apples, the mean ingestion responses by each animal in both contexts were computed. Difference scores were calculated by subtracting mean consumption of apple on treatment days from mean consumption of apple on control days. These scores were then expressed as percentages of control intake and evaluated against a theoretical baseline value of zero% ingestion in a two-way analysis of variance, modified as described in Section 2.1.4. Dunett's procedure[14] was used to isolate significant differences between baseline and treatment means. $P < 0{\cdot}05$ was considered statistically significant.

2.3 Experiment 3

2.3.1 *Subjects*

Twelve European starlings (*S. vulgaris*) were selected from the Monell colony and individually caged (61 × 36 × 41 cm) under 12 : 12 h light : dark cycle. Test materials were prepared as described in Section 2.1.2.

2.3.2 *Procedures*

Prior to the start of the experiment, birds were adapted to an overnight food deprivation regime (1700–0900 h) and familiarized with eating apples. Within one hour of lights-on (0800 h), all birds were presented with fresh apple quarters. After two hours, apple was removed and weighed. From 1000 to 1700 h, all birds had free access to Purina Flight Bird Conditioner (PFBC), crushed oyster shell grit, and tap-water.

After seven days of adaptation, all birds were given two-hour, two-choice tests between pre-weighed apple quarters paired with either OAP or oil on filter paper discs encased in histoprep cassettes. The cassettes were fastened to the lower left and right front wall of cages with plastic ties and apple was placed on the floor directly in front of the cassette. At 1000 h, apple quarters and histoprep cassettes were removed and apples were weighed. At the termination of the study, birds were given free access to PFBC, grit and water until 1700 h.

Subsequently, all birds were tested with apple quarters that had been coated with OAP or oil solution to evaluate directly the effect of direct contact in a comparable study to that used to test OAP's efficacy against prairie voles and deer mice. Apple pieces coated with OAP (10 ml liter^{-1}) solution were placed in the opposite front corners of the birds' cages for two hours, after which consumption was measured. On two consecutive pre-treatment days, birds were presented with two pre-weighed apple pieces and both were paired with the oil-ethanol solution vehicle. Each experiment was performed in duplicate and presentation positions of the OAP and oil solutions were counterbalanced to compensate for potential side preferences.

2.3.3 *Analysis*

To compare avoidance of OAP in histoprep cassettes *vs.* OAP directly applied to apple pieces, mean ingestion responses by each animal in each context were calculated. Difference scores were computed by subtracting mean consumption of OAP-coated apple pieces from consumption of oil-coated apple. These scores were then expressed as percentages of control intake and evaluated against a theoretical baseline value of zero% ingestion. The calculation of the treatment sums of squares was modified as described in Section 2.1.4. Dunett's procedure[14] was used to isolate significant differences between baseline and treatment means. ($P < 0{\cdot}05$).

3 RESULTS

3.1 Experiment 1

OAP decreased apple consumption by voles and mice. This effect was slightly more pronounced for the prairie voles, insofar as all OAP concentrations were repellent. Apple consumption by voles was significantly decreased from theoretical zero% by treatment OAP (0·01–10·0 ml liter^{-1}), $F(1,130) = 21{\cdot}881$, $P < 0{\cdot}00001$ (Fig. 1A). Mice avoided all but the lowest concentration of OAP (0·01–10·0 ml liter^{-1}), ($F(1,130) = 21{\cdot}881$, $P < 0{\cdot}00001$. (Fig. 1B). Total consumption of apples by voles and deer mice did not differ between pre-treatment and treatment days, suggesting that ingestion during the treatment period was largely from oil-coated apples.

3.2 Experiment 2

Voles showed a 9·8 (±5·1)% decrease in consumption when exposed only to the odor of OAP. However, when

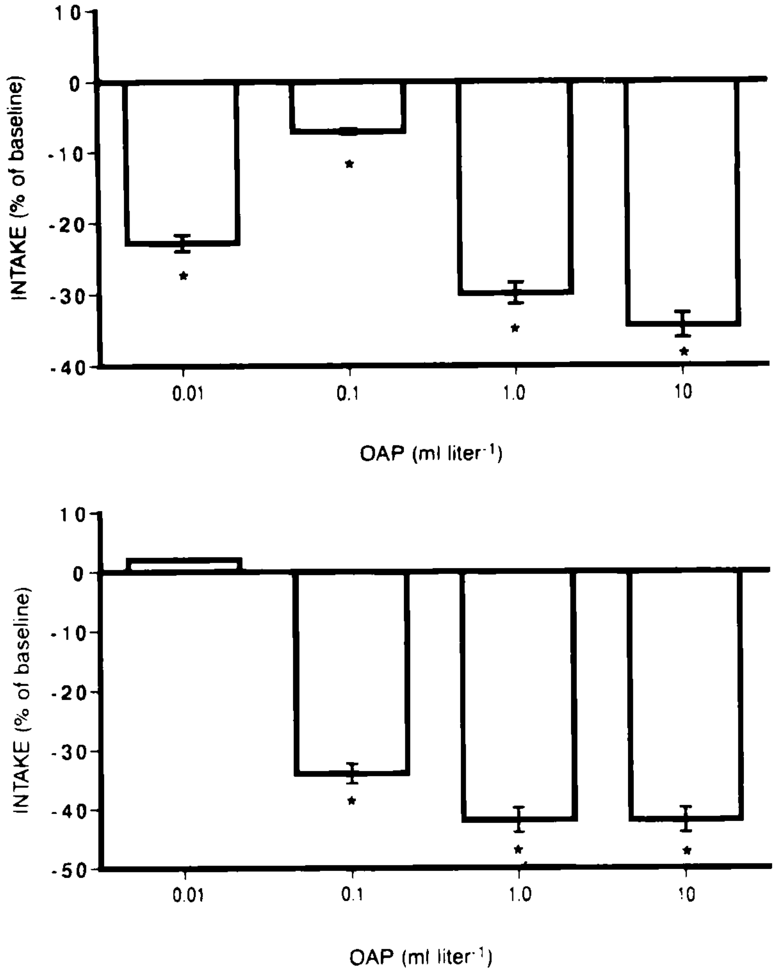

Fig. 1. The effect of OAP solutions on two-hour apple consumption by (A) prairie voles, *Microtus ochrogaster*, and (B) deer mice, *Peromyscus maniculatus*, in two-choice tests. Data are expressed as percentage of control intake. (*) $P < 0{\cdot}05$ compared to theoretical zero% of baseline.

direct contact with OAP-coated apple was permitted, much larger reductions (20·1 ($\pm$5·3)%) were observed. The decreases in apple consumption following both routes of exposure were significantly different from theoretical zero%. $F(1,36) = 14{\cdot}686$, $P < 0{\cdot}0005$ (Fig. 2).

3.3 Experiment 3

When the 10 ml liter^{-1} OAP cue was delivered *via* histoprep cassettes in two-hour, two-choice tests, European starlings decreased consumption of apple by 6·9

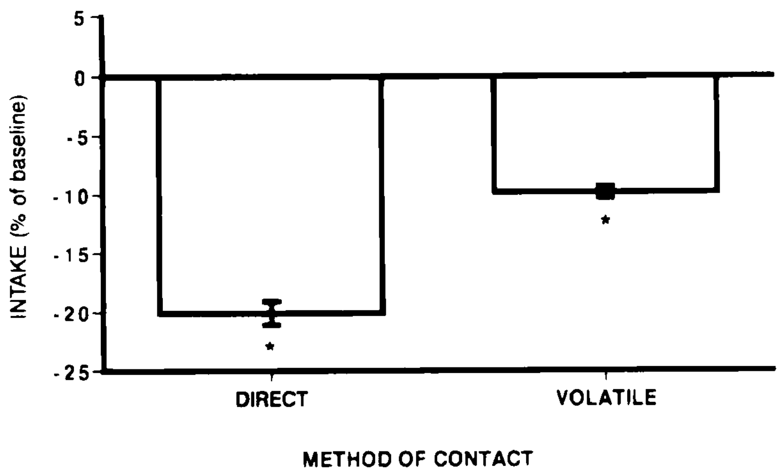

Fig. 2. The effect of the volatile substance, OAP (10 ml liter^{-1}), on two-hour apple consumption by prairie voles, *Microtus ochrogaster* compared to control (oil only) in no-choice feeding trials during direct contact conditions and volatile exposure only conditions. Data are expressed as percentage of control intake. (*) $P < 0{\cdot}05$ compared to theoretical zero% of baseline.

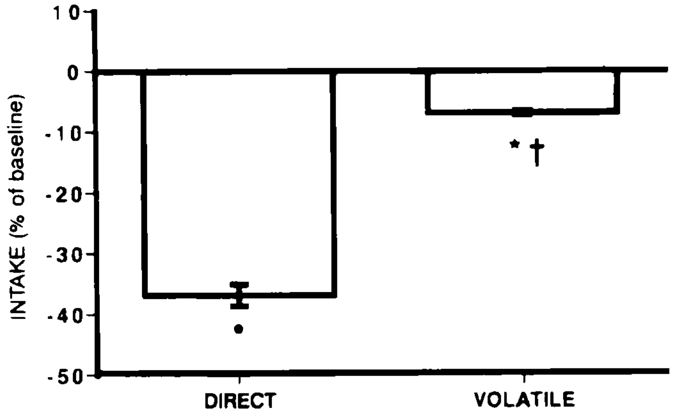

Fig. 3. The effect of the volatile substance, OAP (10 ml liter^{-1}), on two-hour apple consumption by European starlings, *Sturnus vulgaris*, (n = 12) compared to control (oil only) in two-choice feeding trials during the oral contact condition and volatile exposure only condition. Data are expressed as percentage of control intake (*) $P < 0.05$ compared to oil control. (†) $P < 0.05$ compared to direct contact.

($\pm$7·3)%. This reduction was considerably less than the 36·9 ($\pm$10·1)% reduction observed when direct contact with OAP-coated apple was permitted. Decreases in apple consumption by birds following both routes of exposure to OAP were significantly different from zero% consumption, $F(1,44) = 12{\cdot}33$, $P < 0{\cdot}001$ (Fig. 3). Change in apple ingestion by starlings when direct contact of OAP solutions was prevented differed significantly from the amount of apple consumed when the birds had both direct and volatile contact with OAP solutions on the apple pieces, $F(1,44) = 5{\cdot}763$, $P < 0{\cdot}03$, (Fig. 3).

4 DISCUSSION

In the present studies, OAP (10 ml liter^{-1}) coating reduced consumption of a highly preferred food, apple, by deer mice (34·4 ($\pm$10·5)%) and prairie voles (41·9 ($\pm$9·7)%). OAP (10 ml liter^{-1}) coating reduced apple consumption by starlings by 36·9 ($\pm$10·1)% in a comparable experiment. When provided with an alternative food to the OAP-coated apple (i.e oil-coated apple) in two-choice tests, rodents preferentially ingested the alternative choice, maintaining a level of consumption comparable to that achieved during the pre-treatment periods. The reductions in intake observed in these studies are similar to those obtained in previous work.[7,11] Nolte *et al.* found that water consumption by mice (*Mus musculus* L.) was decreased by the addition of OAP.[11] However, the OAP concentrations required to reduce intake to levels not statistically different from zero% were greater than those required to suppress intake in birds comparably.[7]

Methyl anthranilate is a substance that has an odor similar to that of OAP and is structurally related, differing only in the substitution of a ketone for an ester group.[9] Lesioning studies indicate that avoidance of methyl anthranilate is mediated by olfaction and nasal trigeminal chemoreception.[3] This suggests that the volatile characteristics of this substance may be important for its potency as a repellent. The sensory bases for OAP avoidance have not previously been examined. Findings from our studies indicate that the potency of OAP as a repellent agent is diminished when direct contact is not permitted. This suggests the importance of non-volatile cues mediated by oral trigeminal chemoreception and/or taste. Future studies will address whether direct contact with OAP activates chemosensitive receptors in the oral cavity or if retronasal or vomeronasal stimulation mediate the aversive effect of OAP.

5 MANAGEMENT IMPLICATIONS

Few non-lethal chemicals are available for rodent and bird damage control, although they are desirable in a variety of contexts. Those substances that are available are either significantly more effective against one taxon or the other (e.g., methyl anthranilate, capsaicin,[16,17] or unpredictable in performance (e.g. denatonium benzoate).[1] *Ortho*-aminoacetophenone appears to be reliably aversive to mammals and birds. While we are cautious about extrapolating from the laboratory to the field, the present findings have clear practical implications. For example, granular agricultural chemicals can be hazards to birds that ingest them. An additive to these pesticide formulations with bird-repellent capabilities would be especially useful in minimizing these hazards without influencing the effectiveness of the formulation against the target pest. Non-lethal repellents for the combined management of rodent and avian species would be useful in areas that are populated by large numbers of non-target species, such as livestock, companion animals, and humans, to minimize the risk

of accidental poisoning. Additional studies designed to explore practical application of OAP as a vertebrate repellent appear warranted.

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